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Plant and Algal Interference in Bacterial β-D-Galactosidase and β-D-Glucuronidase Assays

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Several commonly occurring freshwater and marine plants and algae were screened for β-D-galactosidase and β-D-glucuronidase activities by using a 60-min enzyme assay based on the hydrolysis by these enzymes of 4-methylumbelliferyl-β-D-galactoside and 4-methylumbelliferyl-β-D-glucuronide, respectively. All freshwater plant extracts tested showed β-D-galactosidase activity several at relatively high levels, and a number also showed β-D-glucuronidase activity. A number of the macroalgae showed no activity of either enzyme, but those showing β-D-galactosidase activity also showed β-D-glucuronidase activity. The majority of microalgae showed some β-D-galactosidase activity, but few showed β-D-glucuronidase activity. Further studies, using the commercial Colilert test and the marine water formulation of Colilert, revealed that 2 of 11 of the microalgal species and several of the plant extracts tested caused positive reactions. It was concluded that several plant extracts and algae could significantly interfere with the detection of coliform bacteria and *Escherichia coli* with the use of rapid assays, on the basis of their production of β-D-galactosidase and β-D-glucuronidase, respectively. The significance of the plant and algal interferences in tests such as Colilert is dependent on the levels of enzymes released under natural conditions, the dilution which they may undergo, and the numbers of algal cells present. This also applies to interferences in rapid enzyme assays. The results of this study demonstrate the potential for interferences in bacterial enzyme assays of waters that contain high plant or algal biomass and suggest the need for masking agents to reduce the contribution of plant and algal enzymes to the response of the assay for the analysis of such waters.

Coliform bacteria have the ability to produce the enzyme β-D-galactosidase which catalyzes the breakdown of lactose into galactose and glucose. β-D-Glucuronidase is the first enzyme in the hexuronide-hexuronate pathway in *Escherichia coli* (17) and is also found in a few *Salmonella* and *Shigella* species (16). These enzymes also hydrolyze a number of chromogenic and fluorogenic substrates. *Ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-galactoside (MU-gal) are hydrolyzed by β-D-galactosidase releasing a colored product, *o*-nitrophenol, and a fluorescent product, 4-methylumbelliferone (MU), respectively. β-D-Glucuronidase hydrolyzes 4-methylumbelliferyl-β-D-glucuronide (MU-glu) which also releases MU.

A number of techniques have been developed for the enumeration of total coliforms, fecal coliforms, and *E. coli*, on the basis of the hydrolysis of one of these substrates by the appropriate enzyme, with subsequent detection of the hydrolysis products (2, 3, 5, 10, 21, 24). The tests are based on the assumption that β-D-galactosidase and β-D-glucuronidase are selective markers for coliforms and *E. coli*, respectively.

The application of enzyme assays to environmental samples relies on the markers being specific and there being no other sources of enzyme activity. The production of β-D-galactosidase and β-D-glucuronidase has been demonstrated in plants (14, 15, 25), in algae (8, 23) and in some noncoliform bacteria (13, 19). Palmer et al. (18) reported the occurrence of 16 false-positive samples of 83 coliform samples tested and 6 false-positive samples of 41 *E. coli* samples tested, using the marine formulation of Colilert (Colilert-MW). Some of the false-positive coliform test results were found to be due to the presence of known β-D-galactosidase-positive, noncoliform bacteria such as *Aeromonas hydrophila*, *Vibrio cholerae* non-O1, and *Kluyvera* species. However, many of the organisms causing the false-positive results remained unidentified. It appeared, therefore, that the evaluation of possible sources of interference is needed.

Apte et al. (4) speculated that some positive results obtained in their rapid fecal coliform test based on the detection of MU, in the absence of culturable fecal coliforms, could be due, in part, to the enzyme activity possessed by marine bacteria, algae, and macroalgal exudates. We therefore examined a number of aquatic plant and algal species for β-D-galactosidase and β-D-glucuronidase activities to assess their possible contribution to false-positive reactions in rapid enzyme-based assays and tests such as Colilert.

MATERIALS AND METHODS

Collection of plant and algal species. Nine of the most commonly occurring Australian marine macroalgae were collected from Warriewood, Cronulla, and Stanwell Park beaches near Sydney, Australia. Seven species of freshwater plants occurring commonly in New South Wales, Australia, were purchased from an aquatic nursery (20). The freshwater macroalga *Cladophora glomerata* was obtained from T. Entwisle, National Herbarium, Royal Botanic Gardens, Melbourne, Australia. The macroalgae and plants were analyzed while fresh, except as otherwise indicated, when they were frozen until required for analysis. Marine and freshwater microalgae were obtained from culture collections at Australian Water Technologies, EnSight, Sydney, and CSIRO Division of Fisheries, Hobart, Australia. Selection of the microalgal species was based on their known occurrence in Sydney's coastal (11)

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or inland (12) waters. Microalgal cultures in log-phase growth were used. Control bacterial cultures for use in the Colilert assay were environmental isolates of *E. coli*, *Pseudomonas aeruginosa*, and a β-D-glucuronidase-negative strain of *E. coli* and were obtained from Australian Water Technologies, Science and Environment.

Reagents. A solution of MU-gal (Sigma Chemical Co., St. Louis, Mo.) was prepared by dissolving 0.0625 g of the compound in 10 ml of dimethylformamide with the assistance of ultrasonication. The resulting solution was made up to a final volume of 250 ml with Milli-Q water (2). The free MU in the solution was removed by passing the MU-gal solution through a Waters Sep-Pak Accell QMA cartridge (Millipore Corp., Bedford, Mass.) which had been conditioned with 10 ml of 0.5 M sodium hydroxide followed by 10 ml of glycine buffer (1.5 g of glycine, 1.2 g of sodium chloride in 50 ml of Milli-Q water, 50 ml of 0.1 M sodium hydroxide, with pH adjustment to 10.2).

A solution of MU-glu was prepared by dissolving 0.05 g of the compound in 100 ml of full-strength Ringer's solution as described above. The MU-glu solution was used in the assay without the removal of free MU.

All reagents for the enzyme assays were sterilized by filtration through a 0.2-μm-pore-size syringe unit filter (Sartorius Gmb*H*, Göttingen, Germany). All seawater used in the assays was filter sterilized (0.2-μm-pore-size filter) and pasteurized at 70°C for 30 min to denature extracellular bacterial enzymes.

Preparation of plants and algae for enzyme assays. The marine macroalgae and freshwater macroalgae and plants were washed several times in sterile seawater and sterile Milli-Q water, respectively. Between 0.1 and 5 g of each marine macroalga and 5 g of each plant and freshwater macroalga were added individually to 100 ml of sterile seawater or Milli-Q water and homogenized for 10 min. The homogenates were centrifuged at 2,000 rpm for 10 min (Jouan CR4-11 centrifuge), and the supernatant was filtered through a sterile 0.45-μm-pore-size syringe unit filter (Sartorius). The filtrates were screened for β-D-galactosidase and β-D-glucuronidase activities.

Cell densities of stock microalgal cultures were determined by counting cells in a hemocytometer by using phase-contrast microscopy. The cultures were diluted to give a final concentration of 10^5 cells per ml in the assay in either sterile seawater (marine species) or sterile Milli-Q water (freshwater species). The algae were then screened for β-D-galactosidase and β-D-glucuronidase activities. All microalgae were examined by microscopy for axenicity.

β-D-Galactosidase and β-D-glucuronidase assays. The β-D-galactosidase assay was carried out by using the method of Apte and Batley (3), with some modifications, as follows: a series of culture tubes (duplicates for each sample) were prepared, each containing 1 ml of PIPES [piperazine-*N*-*N*′-bis(2-ethanesulfonic acid] buffer (giving an assay pH of 7.2) and 5 ml of plant extract or diluted algal culture. Hydrolysis (reagent) blanks (at least three per run of about 20 tubes) were prepared by replacing the 5 ml of plant extract or algal culture with 5 ml of sterile seawater or Milli-Q water. The contents of the tubes were brought to 44.5°C in a water bath, and 4 ml of the MU-gal solution was added to each tube (except the sample blank tubes). Sample blanks (duplicates for each sample) were prepared by substituting the 4 ml of MU-gal solution with 4 ml of sterile Milli-Q water. The tubes were incubated at 44.5°C for 60 min, after which time the reaction was stopped by cooling to room temperature and the addition of 0.4 ml of carbonate buffer to bring the pH to approximately 10.

For the β-D-glucuronidase assay, a series of culture tubes were prepared, each containing 0.1 ml of PIPES buffer and 8 ml of the plant or alga sample. Hydrolysis blanks and sample blanks were again prepared by replacing the sample and the MU-glu with sterile seawater and sterile Milli-Q water, respectively. The tubes were brought to 44.5°C, and 2 ml of the MU-glu solution was added to each tube. The tubes were incubated at 44.5°C for 60 min, after which time the reaction was stopped by cooling to room temperature. No carbonate buffer was added to tubes in the β-D-glucuronidase assay.

The intensity of the fluorescence of the contents of each tube was measured with a Perkin-Elmer LS-5 Luminescence Spectrometer at an excitation wavelength of 375 nm (slit width, 10 nm) and a emission wavelength of 465 nm (slit width, 20 nm). The mean intensities of the fluorescence of the hydrolysis and sample blanks were subtracted from the fluorescence intensities of the plant and algal sample readings. Fluorescence readings were converted to concentrations of MU by using a calibration curve of the spectrometer readings versus standards of known free MU concentrations, prepared in PIPES buffer (pH 7.2). Fluorescence was expressed as the nanomolar concentration of MU liberated by the enzymes. This is an arbitrary scale of activity.

Bacterial contamination. All plant extracts, before and after filtration, and nonaxenic algal cultures were examined for the presence of coliform bacteria by inoculating 1 ml of each extract or culture into lauryl tryptose broth (LTB; Oxoid Ltd., Basingstoke, Hants, United Kingdom) (1) contained in screw-capped tubes with an inverted Durham tube. Duplicate tubes were incubated at 35 ± 0.5°C. They were examined for visible signs of growth in the broth and collection of gas in the Durham tube after 24 ± 2 h and again after 48 ± 3 h. The absence of growth and gas after 48 h was considered a negative reaction indicating the absence of coliform bacteria.

Enzyme leakage from plants. Samples of *Lemna* sp., *Juncus usitatus*, and *Myriophyllum* sp. were washed thoroughly with tap water and rinsed with sterile Milli-Q water. Sterile Milli-Q water was placed in each of three sterile beakers (1 liter). A plant was placed in each of the beakers and covered loosely with a polythene bag to prevent contamination while allowing the plant exposure to light and gaseous exchange. The contents of the beakers were mixed thoroughly with a sterile pipette, and approximately 20 ml of the water was withdrawn. The water was analyzed for β-D-galactosidase activity, and 1 ml was inoculated into tubes of LTB to detect the presence of coliform bacteria. The beakers were stored at room temperature close to the laboratory window, and samples of the water were withdrawn periodically for analysis for the duration of the experiment, about 3 weeks.

Plant and algal interferences in Colilert. Those species of plants and algae showing high β-D-galactosidase or β-D-glucuronidase activities and a number of species showing low activities, for comparison, were screened for positive reactions in the commercially available presence-absence format of the Colilert assay. Conventional Colilert (Environetics, Inc., Branford, Conn.) was used for freshwater species, and Colilert-MW (Environetics, Inc.), the marine water formulation of the assay, was used for the marine species. Triplicate Colilert tubes were inoculated with either 10 ml of plant extract (5 g/100 ml) or 10 ml of algal sample diluted with sterile Milli-Q water or seawater to give a final concentration of 1.0×10^5 algal cells per ml. Colored plant extracts were diluted appropriately to reduce the intensity of the color. Sterile Milli-Q water or seawater (10 ml) inoculated into 18- to 24-h cultures of *P. aeruginosa*, *E. coli*, and a β-D-glucuronidase-negative strain of *E. coli* was used in triplicate control tubes for each assay. All

TABLE 1. Interferences by freshwater plant and macroalgal extracts in enzyme assays[a]

Species	Fluorescence (nM MU)[b]	
	MU-gal	MU-glu
Typha orientalis	71,481 (16)	89
Juncus usitatus	1,337 (159)	17
Myriophyllum sp.	87 (ND)	ND
Lemna sp.	441 (84)	ND
Nyphaea capensis	23 (ND)	ND
Salvinia molesta	6,319 (ND)	ND
Eichhornia crassipes	1,243 (71)	16
Cladophora glomerata	1,682 (ND)	5

[a] Extract concentration, 5 g/100 ml.
[b] Values in parentheses are the fluorescence of the extract diluted 1:50, except. *T. orientalis* was diluted 1:3,200 and *S. molesta* was diluted 1:2,500. ND, not detected.

TABLE 2. Interferences by marine macroalgal extracts in enzyme assays

Species[a]	Extract concn (g/100 ml)	Fluorescence (nM MU)[b]	
		MU-gal	MU-glu
Caulerpa filiformis	5	9,522 (1,904)	24 (5)
Zonaria sp.	5	4 (1)	6 (1)
Dictota dichotoma	0.25	ND	ND
Enteromorpha linza	0.1	4 (40)	21 (210)
Sargassum sp.	0.1	ND	ND
Phyllospora comosa	0.1	ND	ND
Hormosira banksii	5	ND	ND

[a] All stored frozen and thawed for assay.
[b] Values in parentheses were normalized per g of extract. ND, not detected.

tubes were incubated at 35°C for 24 h, after which they were examined by eye for the presence of a yellow coloration and fluorescence under a UV lamp. Algal cells in Colilert-MW after 1 and 24 h, and in the 60-min β-D-galactosidase assay at 44.5°C after 1 h, were examined under the microscope.

RESULTS AND DISCUSSION

Plant and algal interferences in rapid β-D-galactosidase and β-D-glucuronidase assays. Enzyme activities, in terms of the concentration of MU liberated by β-D-galactosidase and β-D-glucuronidase enzymes, in several freshwater plant species and a freshwater macroalgal species are given in Table 1. For many of the species tested, the values obtained for diluted plant extracts were much higher than expected for the respective dilutions, indicating that at high concentrations of plant extract there may be a quenching of the fluorescence of the MU, possibly by plant pigments (6, 22).

All species of freshwater plants tested exhibited β-D-galactosidase activities, and several exhibited β-D-glucuronidase activities, at sufficiently high concentrations to be detected in the assay. In comparison with the fluorescence readings obtained in the β-D-galactosidase assay for the plant extracts, typical fluorescence readings obtained for sewage-contaminated seawater samples containing approximately 100 fecal coliforms per ml are in the range of 300 to 1,000 nM MU (3). Fluorescence readings given by approximately 100 CFU of *E. coli* per ml were around 50 nM MU in the β-D-glucuronidase assay. Sterile seawater, sterile Milli-Q water, and pure cultures of *P. aeruginosa* (10^8 CFU/ml) gave readings below the limit of detection (<2 nM) for both β-D-galactosidase and β-D-glucuronidase assays.

Three of seven species of marine macroalgae tested exhibited some enzyme activity (Table 2). Surprisingly high β-D-galactosidase activity can be seen in the marine macroalga *Caulerpa filiformis*. Three marine macroalgae exhibited some β-D-glucuronidase activity. It is acknowledged, however, that the concentrations of plant material screened here for enzyme activities are relatively high compared with those found in the surfline.

All filtered and unfiltered plant and macroalgal extracts were free of bacteria capable of growth and gas production in LTB at 35°C within 48 h, with the exception of the unfiltered extract of *Cladophora glomerata*. The filtered extract of this species contained no coliforms, but there was the possibility of the presence of extracellular bacterial enzymes, and, therefore, we could not confidently attribute the measured enzyme activity solely to *Cladophora glomerata*.

Eight of 9 species of freshwater microalgae (Table 3) and 9 of 13 species of marine microalgae (Table 4) tested exhibited some β-D-galactosidase activity. Only two of the nine species of freshwater microalgae and 6 of 13 marine microalgae tested exhibited any β-D-glucuronidase activity. Only eight of the species used were axenic. Microscopic examination showed that some of the other species were contaminated with bacterial cells, although these bacteria did not have the ability to grow or produce gas in LTB at 35°C within 48 h. The β-D-galactosidase and β-D-glucuronidase activities were not associated with a particular taxonomic group nor did they appear to be related to cell size.

The cell densities used in these experiments for most species were at least 10 times higher than maximum cell densities reached in bloom conditions. Algal blooms with cell densities reaching 10^4 cells per ml are a common occurrence in coastal waters off Sydney in spring and summer (11). Attempts to predict the fluorescence liberated by the enzymes at more realistic cell densities were made by using values reported in the literature and the experimental data presented in Tables 3 and 4. *Skeletonema pseudocostatum* and *Gymnodinium* spp. occur in Sydney coastal waters at typical maximum cells densities of 100/ml and 700/ml, respectively (11). At these cell densities, it is predicted that these two species would produce concentrations of β-D-galactosidase of 3 and 40 nM MU, respectively, and that *Gymnodinium* spp. would also produce β-D-glucuronidase at a concentration of 10 nM MU. Enzyme activities of the other marine microalgae tested would not be sufficiently high to be detected.

Plant and algal interferences in Colilert and Colilert-MW. All marine macroalgal species, one freshwater plant species, and two marine microalgal species tested resulted in positive β-D-galactosidase reactions in Colilert after 24 h (Table 5). There were, however, no positive reactions for the β-D-glucuronidase part of the assay from any species tested. The control bacterial species gave characteristic reactions in both Colilert and Colilert-MW. *E. coli* was positive in both β-D-galactosidase and β-D-glucuronidase parts of the assay, *P. aeruginosa* was negative in both β-D-galactosidase and β-glucuronidase parts of the assay, and β-D-glucuronidase-negative *E. coli* was positive in the β-D-galactosidase and negative in the β-D-glucuronidase parts of the assay. Sterile Milli-Q water and seawater gave no positive reactions. A number of the plant and marine macroalgal extracts were slightly colored even after dilution, making the tubes for the presence of *o*-nitrophenol somewhat difficult to read; so, in accordance with the Colilert instructions for use, the colors of the Colilert tubes were

TABLE 3. Interferences by freshwater microalgae in enzyme assays

Family and species	Strain no.[a]	Cell diam or dimensions (μm)	Cell density (cells/ml)	Fluorescence (nM MU)	
				MU-gal	MU-glu
Chlorophyceae					
Cosmarium sp.		40	2.4×10^4	3,359	ND[b]
Scenedesmus obliquus		~6	1.0×10^5	1,601	ND
Ankistrodesmus sp.		50×6[c]	1.0×10^5	107	ND
Chlamydomonas reinhardii[d]	CS-51	5	1.0×10^5	10	12
Chlorella protothecoides[d]	CS-41	3	1.0×10^5	4	ND
Selenastrum capricornutum[d]	ATCC 22662	4	1.0×10^5	3	ND
Chlorella vulgaris[d]	CS-42	3	1.0×10^5	2	ND
Cyanophyceae					
Microcystis sp.		~3	1.0×10^5	112	ND
Anabaena cylindrica	CS-53	5–10 × 2–5[c]	1.0×10^5	ND	11

[a] CS, CSIRO Division of Fisheries Microalga Culture Collection, Hobart, Australia. ATCC, American Type Culture Collection, Rockville, Md.
[b] ND, not detected.
[c] Cell dimensions expressed as length by width.
[d] Axenic culture.

compared with the colors of the sample with no Colilert added. Subsequently, positive reactions were obtained for *Phyllospora comosa* and *Plocomium* sp. Two of the marine macroalgal species, *Sargassum* sp. and *Phyllospora comosa*, gave positive reactions in Colilert-MW but did not show any appreciable β-D-galactosidase activity in the rapid assay. This is probably due, in part, to differences in concentrations of the enzymes found in algae collected at different sites and times of year and to differences in extract concentrations used because of the need to dilute the colors of some extracts.

Within the first hour of incubation, the only algal species which appeared unaffected in the Colilert-MW reaction was *Dunaliella tertiolecta*. The other five species showed evidence of cytoplasm shrinkage or cell lysis. In the rapid β-D-galactosidase assay, run in parallel with Colilert-MW, all six species tested appeared to be stressed. *Dunaliella tertiolecta* and *Cryptomonas maculata* were intact but granular in appearance and nonmotile. The other species were largely lysed. Cells in sample blanks (no MU-gal) were in a state similar to that of their counterparts in the assay tubes, suggesting that it was probably temperature, rather than an inhibitory effect of MU-gal, which had stressed the cells. Only cells of *Dunaliella tertiolecta* were intact after 24 h in Colilert. These were granular in appearance and had lost their motility.

Release of β-D-galactosidase from plants. Figure 1 shows the β-D-galactosidase activities in water samples over a period of 3 weeks. Within 24 h, β-D-galactosidase had been released from the three plants into the water. At this point, no coliform

TABLE 4. Interferences by marine microalgae in enzyme assays

Family and species	Strain no.[a]	Cell diam or dimensions (μm)	Cell density (cells/ml)	Fluorescence (nM MU)	
				MU-gal	MU-glu
Bacillariophyceae (diatoms)					
Skeletonema pseudocostatum	CS-76	7–12 × 5–7[b]	1.5×10^5	3,877	13
Chaetoceros gracilis	CS-176	5–8	1.5×10^5	ND[c]	ND
Asterionella glacialis	CS-135	10–15 × 2–8[b]	5.2×10^4	277	19
Nitzschia closterium[d]	CS-5	40×2[b]	1.3×10^5	ND	ND
Chlorophyceae, Dunaliella tertiolecta[d]	CS-175	6	1.5×10^5	638	ND
Cryptophyceae, Cryptomonas maculata	CS-85	~9	1.5×10^5	237	4
Dinophyceae					
Prorocentrum gracile	CS-80	40×15[b]	7.2×10^5	1,785	49
Gymnodinium sanguineum	CS-35	80–100 × 40–60[b]	2.5×10^3	143	34
Prasinophyceae					
Tetraselmis sp.[d]	CS-87	9	1.5×10^5	238	ND
Pyramimonas cordata	CS-140	8	1.5×10^5	30	5
Micromonas pusilla	CS-86	2	1.5×10^5	ND	ND
Prymnesiophyceae					
Isochrysis sp.[d]	CS-177	~5	2.0×10^5	22	ND
Emiliania huxleyi	CS-57	~5	1.5×10^5	ND	ND

[a] CS, CSIRO Division of Fisheries Microalga Culture Collection, Hobart, Australia.
[b] Cell dimensions expressed as length by width.
[c] ND, not detected.
[d] Axenic culture.

TABLE 5. Plant and algal enzyme activities in the Colilert and Colilert-MW tests

Species or sample	Cell density (cells/ml) or extract concn[a]	Colilert reaction[b] β-gal	Colilert reaction[b] β-glu
Freshwater			
Typha orientalis	5[a]	+	−
Juncus usitatus	5[a]	−	−
Lemna sp.	5[a]	−	−
Salvinia molesta	5[a]	−	−
Nyphaea capensis	5[a]	−	−
Scenedesmus obliquus	1.0×10^5	−	−
Ankistrodesmus sp.	7.8×10^4	−	−
Chlamydomonas reinhardtii	1.0×10^5	−	−
Anabaena cylindrica	1.0×10^5	−	−
Cosmarium sp.	1.3×10^4	−	−
Controls			
Sterile Milli-Q water		−	−
P. aeruginosa	3.4×10^6	−	−
E. coli	5.0×10^6	+	+
E. coli (β-D-glucuronidase negative)	$\sim 10^6$	+	−
Marine			
Caulerpa filiformis	5[a]	+	−
Sargassum sp.	5[a]	+	−
Phyllospora comosa	2.5[a]	+	−
Ecklonia radiata	5[a]	+	−
Plocomium sp.	2.5[a]	+	−
Nitzschia closterium[c]	1.0×10^5	−	−
Asterionella glacialis[c]	1.0×10^5	−	−
Dunaliella tertiolecta[c]	1.0×10^5	−	−
Cryptomonas maculata[c]	1.0×10^5	+	−
Skeletonema pseudocostatum[c]	1.0×10^5	+	−
Controls			
Sterile seawater		−	−
P. aeruginosa	3.8×10^6	−	−
E. coli	5.6×10^6	+	+
E. coli (β-D-glucuronidase negative)	$\sim 10^6$	+	−

[a] Extract concentration is expressed in g/100 ml.
[b] β-gal, β-D-galactosidase; β-glu, β-D-glucuronidase.
[c] Species examined under microscope after 1 and 24 h.

bacteria were detected. After 5 days, decomposition of *Myriophyllum* sp. and *Lemna* sp. was accompanied by an increase in numbers of bacteria which were capable of utilizing lactose at 35°C within 24 h. This indicates that there was a low, undetectable number of contaminating bacteria originally present which, possibly on release of nutrients from the decomposing plants, were able to multiply and significantly contribute to the β-D-galactosidase activity in the waters. The β-D-galactosidase activities continued to increase until day 10 when the rate of increase slowed, and coliforms were present for the remainder of the experimental period. No coliforms were detected in the water with *Juncus usitatus*, and no decomposition was observed. However, β-D-galactosidase activity was detected and remained constant at this level throughout the 3-week period.

β-D-Galactosidase and β-D-glucuronidase have been identified in numerous terrestrial plant species (14, 25) and have been found in different parts of plants (15). An extensive search of the literature revealed that the number of reports

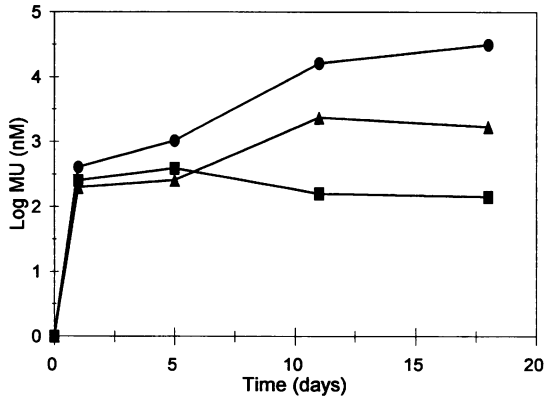

FIG. 1. Leaching of β-galactosidase from freshwater plants. Plants were incubated at room temperature (approximately 20°C) in sterile Milli-Q water at pH. ■, *Juncus usitatus*; ●, *Myriophyllum* sp.; ▲, Lemna sp.

describing β-D-galactosidase or β-D-glucuronidase activities in algae are far fewer than those for plants. β-D-Galactosidase, but not β-D-glucuronidase, has been detected in some strains of *Prototheca* sp. (8) and in *Acetabularia mediterranea* (23), and Chróst et al. (9) found β-D-galactosidase activity in the algal fraction of lake water but attributed this to the presence of bacteria and incomplete separation of algal and bacterial fractions by differential filtration. In contrast, the production of inhibitors of bacterial β-D-galactosidase production by *Chlamydomonas plethora* has been reported (7). As far as we are aware, the present study is the first detailed investigation into β-D-galactosidase and β-D-glucuronidase activities in algae.

It is not known where in the algal cell the β-D-galactosidase and β-D-glucuronidase are located. β-D-galactosidase has been detected in both the cytoplasm and the cell wall of the unicellular green alga *Acetabularia* (marine) sp. (23). We used microalgae in the mid to late log phase with the intention of maximizing enzyme activity, in agreement with the findings of Vanden Driessche et al. (23), who found maximum β-D-galactosidase activity towards the end of the log phase in an *Acetabularia* sp.

It can be concluded that the presence of some alga and plant material at high concentrations may significantly interfere with the detection and enumeration of coliform bacteria by methods which are based on the production of β-D-galactosidase and β-D-glucuronidase enzymes. For algae, this will depend on the numbers of cells present and may be significant when there are algal blooms. The significance of the production of β-D-galactosidase and β-D-glucuronidase enzymes by aquatic plants and macroalgae is less easily interpreted, as it is difficult to estimate concentrations at which plants may realistically release the enzymes into the environment. Our attempts to assess the release of β-D-galactosidase from growing and decomposing plants showed that considerably high concentrations of enzyme leached into the surrounding waters. However, these observations were made in a closed system and did not take into consideration the enormous dilution which the enzymes would undergo, particularly in the ocean or a flowing river. Conditions in the experiment may more accurately

reflect the situation in a stagnant pond. It should be emphasized, therefore, that when collecting water samples for bacteriological analysis using enzyme technologies, consideration of the presence of decomposing and growing plant material is advisable.

The present study has highlighted that when monitoring environmental waters for bacteria by using enzyme assays, one may not be able to attribute the assay response solely to bacteria and that, when developing rapid enzyme assays, this must be taken into consideration. In particular, the combined enzyme activities of algae, plant material, and noncoliform bacteria may yield false-positive results or make interpretation of the Colilert test somewhat difficult. Studies of ways of masking or otherwise removing the contribution of plant and algal enzymes to assay responses are currently underway in our laboratory.

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